Consumer Method To Control *Salmonella* and *Listeria* Species in Shrimp

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ABSTRACT

The purpose of this study was to determine whether the current consumer method of boiling shrimp until floating and pink in color is adequate for destroying *Listeria* and *Salmonella*. Shrimp samples were submerged in bacterial suspensions of *Listeria* and *Salmonella* for 30 min and allowed to air dry for 1 h under a biosafety cabinet. Color parameters were then measured with a spectrophotometer programmed with the CIELAB system. Twenty-four shrimp samples were divided into groups (days 0, 1, or 2) and stored at 4°C. The samples were treated by placing them in boiling water (100°C) on days 0, 1, and 2. The shrimp were immediately removed from the boiling water once they floated to the surface, and color parameters were measured. Bacterial counts were determined, and the log CFU per gram was calculated. The effect of sodium tripolyphosphate on the color change of cooked shrimp also was determined. Initial bacterial counts on shrimp after air drying were 5.31 ± 0.14 log CFU/g for *Salmonella* Enteritidis, 5.24 ± 0.31 log CFU/g for *Salmonella* Infantis, 5.40 ± 0.16 log CFU/g for *Salmonella* Typhimurium, 3.91 ± 0.11 log CFU/g for *Listeria innocua*, 4.45 ± 0.11 log CFU/g for *Listeria monocytogenes* (1/2a), and 3.70 ± 0.22 log CFU/g for *Listeria welshimeri*. On days 0, 1, and 2, all bacterial counts were reduced to nondetectable levels for shrimp samples that floated. The average time for shrimp to float was 96 ± 8 s. The bacterial counts remained at nondetectable levels (<10 log CFU/g) during refrigerated (4°C) storage of cooked shrimp for 2 days. The redness, yellowness, and lightness were significantly higher (P < 0.0001) for the cooked shrimp than for the uncooked shrimp on all days tested. The standard deviation for redness in the cooked shrimp was large, indicating a wide range of pink coloration on all days tested. The results suggest that boiling shrimp until they float will significantly reduce *Listeria* and *Salmonella* contamination, but color change is not a good indication of reduction of these pathogens because of the wide natural color variation.

Heat treatment, usually in the form of cooking, plays a major role in the safety and sensory acceptance of seafood. Cooking is defined as “the application of heat to a food to modify raw product properties in order to meet sensory expectations of consumers and to reduce its microbial load, which improves its safety and may extend its shelf life” (10). A popular method of cooking shrimp is boiling. Consumers will often boil shrimp until they float to the surface of the water. Additionally, a common method used by consumers to determine doneness when cooking is to observe the color change of the cooked product. When boiling shrimp, the color change of the shrimp from gray to pink is associated with the shrimp being thoroughly cooked. This color change is used by consumers for safety evaluation and is associated with sensory acceptance. Improper cooking and storage contribute to a large number of foodborne illness outbreaks. Therefore, it is necessary to test the reliability of these widely used cooking methods and to provide guidelines for consumers to ensure the safety of their food.

Some additives in shrimp inhibit the pink color change, which can result in a safety hazard to consumers. Phosphates, particularly sodium tripolyphosphate, are commonly added to shrimp to avoid excess moisture loss during processing, distribution, storage, and preparation, which in turn results in maintenance of the quality and consumer acceptance of the shrimp (7). However, when overused, this ingredient may cause excess retention of water, which can result in adulteration of products and can prevent color change of shrimp during cooking. Further research is needed on the effect of phosphate treatment on the pink color change of shrimp during cooking.

Various factors such as thermal resistance of microorganisms and storage conditions can also affect the cooking time and temperature needed to ensure destruction of pathogens in a product. In a detailed review of the literature, Wan Norhana et al. (17) found that there was very limited information on cooking to destroy *Salmonella* in shrimp. This lack of information is exacerbated by the fact that the incidence of *Listeria* and *Salmonella* is much greater in imported cultured shrimp (1–4, 8, 9, 11–13), and the incidence of *Salmonella* is higher at the retail level that at the processing or wholesale facilities. Thus, temperature abuse seems far more likely at retail outlets (17).

At 2 to 4°C, certain organisms such as *Listeria monocytogenes* can grow and multiply (2, 6, 14), and...
growth under cold stress can promote development of heat resistance in some pathogens. Products may be stored in the refrigerator after cooking by the consumer, and some distributors sell already cooked, ready-to-eat seafood products that are stored at refrigeration temperatures. As a result, the risk of growth or contamination during refrigerated storage of previously cooked shrimp products must be evaluated. All of these factors must be considered when determining proper processing and storage conditions for control of Listeria and Salmonella in shrimp and shrimp products. Generally, consumers store shrimp at refrigerator temperatures (2 to 4 °C) for up to 2 days before cooking, as recommended by the U.S. Food and Drug Administration (FDA) (16).

The aim of this study was to determine whether the shrimp cooking method used commonly in U.S. households of boiling until floating and pink in color is adequate for reducing Listeria and Salmonella to nondetectable levels. This study was conducted by determining the thermal log reduction of Listeria and Salmonella on the surface of whole shrimp necessary to reach nondetectable levels and by determining a correlation between pathogen reduction to nondetectable levels and the degree of color change after shrimp were boiled until they floated.

MATERIALS AND METHODS

Culture preparation. Salmonella Enteritidis (13076), Salmonella Infantis (Centers for Disease Control and Prevention [CDC]), Salmonella Typhimurium (ATCC 14028), Listeria innocua (Lm F4248, CDC), Listeria monocytogenes (1/2a, Lm F4263, CDC), and Listeria welshimeri (ATCC 35897) were obtained from the Department of Food Science frozen culture collection at Louisiana State University (Baton Rouge). Frozen cultures were thawed, 10 μl of each Salmonella serotype was suspended in 9 ml of brain heart infusion (BHI; Acumedia Manufacturers, Lansing, MI) broth, and 10 μl of each Listeria serotype was suspended into 9 ml of tryptic soy broth (TSB; BD, Franklin Lakes, NJ). The cultures were incubated at 37 °C for 16 h to achieve stock cultures that were approximately 10⁶ to 10⁹ CFU/ml. After 16 h, all cultures were transferred to slants and maintained at room temperature (23 °C) for future use. Salmonella and Listeria subcultures were made by suspending a loopful (10 μl) of cells from the agar slants in 9 ml of BHI broth for Salmonella and 9 ml of TSB for Listeria and incubated for 16 h at 37 °C. This resulted in an approximately 10⁸ CFU/ml working culture to be used for inoculation.

Sample preparation. Shrimp samples were purchased from a local seafood market on the day of delivery (Maxwell’s Market, Baton Rouge, LA) and consisted of large Gulf Coast shrimp (21 to 30 per lb [46 to 66 per kg]) with the shells and heads on. Individual shrimp weighed approximately 16 ± 2 g. On the day of inoculation, the samples were washed thoroughly with tap water and then with sterile distilled water immediately before inoculation. Fifty milliliters of respective 16-h cultures was added to a sterile container holding 500 ml of sterile 0.1% phosphate-buffered saline (PBS) solution to make an approximately 10⁶ CFU/ml bacterial suspension. The shrimp samples were soaked in the culture solution for 30 min and then allowed to air dry for 1 h in a biosafety cabinet. This procedure was followed for each of the six bacterial isolates used in this study. The final level of the bacteria was 3.5 to 5.5 log CFU/g. After inoculation, the shrimp samples were randomly picked and separated into three time periods: day 0, 1, or 2. Day 1 and day 2 sample groups were stored at 4 °C before heat treatment.

Thermal destruction procedures. The day of inoculation was day 0. Immediately after surface inoculation and drying, individual shrimp samples were placed into a boiling water bath (100 °C) in an immersible 6-qt (5.6-liter) multicooker (National Presto Industries, Eau Claire, WI) containing 1 liter of tap water and were removed immediately once they floated to the surface of the water and were pink in color. The time it took for the shrimp to float to the surface of the water was recorded. A traceable thermometer with a digital probe (Control Company, Friendswood, TX) was used to monitor the internal shrimp temperature. The thermometer probe was inserted at the cold spot of the shrimp, i.e., the thickest part, which is between the first and second abdominal segment, and monitored individually. Surface temperature of each shrimp was measured with a ThermoTrace 15036 infrared laser thermometer (Delta Trak, Pleasanton, CA). After the temperatures were recorded, the heated samples were immediately transferred with sterile tongs to Whirl-Pak filter sample bags (Nasco, Salida, CA). The bags were then submerged in an ice-cold water bath to stop the cooking process.

Enumeration of bacteria. The shrimp samples were weighed, and an equal (wt/vol) amount of PBS was added to each Whirl-Pak bag. The bag contents were then homogenized in a Lab-Blender 400 Stomacher (Tekmar Co., Cincinnati, OH) at normal speed for 1 min. Under aseptic conditions, decimal dilutions of each sample were prepared, and the dilutions were plated in triplicate on xylose lysine deoxycholate (Remel, Thermo Fisher Scientific, Lenexa, KS) plates for Salmonella and on Oxford Listeria agar (Acumedia) plates that had been modified by adding Listeria selective enrichment supplement (Acumedia) for Listeria species. Plates were incubated overnight at 37 °C. After incubation, colonies were counted and the log CFU per gram was calculated for each plate.

On days 1 and 2, the samples designated day 1 and day 2 were removed from refrigerated storage, and the above procedures for heat treatment and enumeration were followed. All experiments were conducted three times.

Time needed for pathogens to reach nondetectable levels in shrimp. This study was performed to determine the time point during boiling when the pathogenic bacteria were killed. The average time to floating for individual shrimp was calculated as 80 to 90 s, depending on the size of the shrimp. To determine the destruction of the bacteria on the shrimp during boiling, shrimp were evaluated at five time points: 0, 25, 50, 75, and 90 s. For this study, the previously described methods for sample preparation were followed except using only Salmonella Typhimurium and L. monocytogenes. The individual shrimp were then placed in the boiling water bath and removed at the various time points, and the bacteria on each shrimp were enumerated. This study was conducted three times.

Consumer cooking portion study. Because consumers are unlikely to boil and eat just one individual shrimp at a time, a cooking portion study was performed. In this study, a more common size of shrimp was used; 1 lb (0.45 kg) of large (21 to 30 per lb) shrimp with shells and heads (Maxwell’s Market). The same procedures for sample preparation as used in the original study were followed, except only the most pathogenic and resistant strains, Salmonella Typhimurium and L. monocytogenes, were evaluated. Typical cookbooks recommend boiling 1 lb of shrimp in 4 cups (0.9 liters) of water; therefore, 4 cups of water was used to boil the shrimp in this
study (5). The water was brought to a boil (100°C) in the water bath, and then 1 lb of shrimp was added. The time to floating was recorded. The samples were then transferred to sample bags, and bacteria were enumerated. The study was conducted three times.

**Determining bacterial growth during refrigerated storage of cooked shrimp.** This study was conducted to determine whether bacterial counts would remain at nondetectable levels after the shrimp were heat treated until floating and then stored at 4°C. Shrimp samples were inoculated as described and separated into three groups: days 0, 1, and 2. On day 0, all shrimp samples were placed into boiling water and cooked until floating. Bacterial counts were determined on days 0, 1, and 2 as described above.

**Degree of color change after shrimp were boiled until floating.** The color of the cooked and uncooked shrimp shell was measured with a spectrophotometer (CM-508d series, Minolta, Osaka, Japan) programmed with the CIELAB system for L* (lightness), a* (red to green), and b* (yellow to blue) values. The color of cooked shrimp was evaluated immediately after the heat treatment. Before taking color measurements, the device was calibrated by taking readings from the surroundings and then from a white color standard (L* = 97.21, a* = 0.55, and b* = 2.12). Three color readings were taken at the same spot for each shrimp, i.e., the thickest part, between the first and second abdominal segments.

**Determining the effect of no added sodium tripolyphosphate on the color change of shrimp during cooking.** The methods used for this study were the same as used in the original study, and the color analysis was the same as described above. The difference was that the shrimp was purchased directly from a local Louisiana fisherman (AnnaMarie’s Seafood, Houma, LA), who had not applied any sodium tripolyphosphate to the shrimp.

**Statistical analysis.** Differences in survival of all bacterial inocula after the shrimp were boiled until floating were analyzed for significance with Student’s t test after a one-way analysis of variance (ANOVA) JMP-IN (version 9.0, SAS Institute, Cary, NC). Differences were considered significant at \( P < 0.05 \). Color was analyzed by taking the mean of the color readings for each temperature treatment per replicate and submitting these data to a one-way ANOVA.

**RESULTS**

**Thermal reduction after boiling until floating.** The results from the experiment in which the shrimp were boiled until floating revealed that after heat treatment the bacterial load was reduced to nondetectable levels (<10 CFU/g) for *Salmonella* and *Listeria* on days 0, 1, and 2. Table 1 shows the results for the thermal reduction of *Salmonella* Enteritidis, *Salmonella* Infantis, and *Salmonella* Typhimurium. The initial mean inoculum level for *Salmonella* was 5.3 log CFU/g, which was reduced postcooking to nondetectable levels. Table 2 contains the results for the thermal reduction of *L. innocua*, *L. monocytogenes*, and *L. welshimeri* after boiling until floating. The initial mean inoculum levels for the *Listeria* species were 3.5 to 4.5 log CFU/g, which were reduced postcooking to nondetectable levels.

After shrimp were boiled until floating, the average internal temperature was 68.61 ± 2.29°C, with a minimum of 65.2°C, and the average surface temperature was 76.67 ± 4.9°C. The variation in both the internal and surface temperatures was due to the variation in the size of the shrimp. The average size of the shrimp was 16.09 ± 2 g. The temperature had a direct relationship with size, and larger shrimp reached a higher internal temperature. Larger shrimp also took longer to float to the surface of the water. The average time it took the shrimp to float to the surface when boiled was 96 ± 8 s.

**TABLE 1. Thermal reduction of Salmonella Typhimurium, Salmonella Infantis, and Salmonella Enteritidis counts on shrimp after they were boiled until floating**

<table>
<thead>
<tr>
<th>Salmonella inoculum</th>
<th>Cooked</th>
<th>Uncooked</th>
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<tr>
<td></td>
<td>Day 0</td>
<td>Day 1</td>
</tr>
<tr>
<td>Typhimurium</td>
<td>ND(^a)</td>
<td>ND</td>
</tr>
<tr>
<td>Infantis</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>Enteritidis</td>
<td>ND</td>
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\(^a\) ND, nondetectable (<10 CFU/g).

**TABLE 2. Thermal reduction of Listeria welshimeri, L. monocytogenes, and L. innocua counts on shrimp after they were boiled until floating**

<table>
<thead>
<tr>
<th>Listeria inoculum</th>
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<th>Uncooked</th>
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<tr>
<td></td>
<td>Day 0</td>
<td>Day 1</td>
</tr>
<tr>
<td>L. welshimeri</td>
<td>ND(^a)</td>
<td>ND</td>
</tr>
<tr>
<td>L. monocytogenes</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>L. innocua</td>
<td>ND</td>
<td>ND</td>
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\(^a\) ND, nondetectable (<10 CFU/g).
Time needed for pathogens to reach nondetectable levels in shrimp. In the time point study, L. monocytogenes and Salmonella Typhimurium on shrimp were reduced to nondetectable levels after boiling in water at 100°C for 75 s (Fig. 1). At this time point, the internal temperature reached an average of 68.25 ± 3°C, very similar to the 68.61 ± 2°C recorded in the thermal reduction experiment. At 90 s, the average internal temperature was 69.95 ± 1°C. No significant change in temperature occurred between 75 and 90 s.

Cooking portion study. Boiling of 1 lb of shrimp until they floated to the surface was adequate for eliminating the bacteria on the shrimp. The time to floating for 1 lb was 105 ± 2 s in contrast to 96 ± 8 s for an individual shrimp.

Bacterial growth during refrigerated storage. Table 3 shows the growth of Salmonella in shrimp after they were boiled until floating on day 0 and stored under refrigeration; no bacteria were detected after day 1 and day 2 of refrigerated storage. Initial inoculation counts for Listeria species were 4.29 to 5.48 log CFU/g, and all bacterial counts were reduced to nondetectable levels postcooking.

The degree of color change after boiling shrimp until floating. Color readings were taken before and after cooking for each shrimp and were analyzed after cooking. The color analysis (Fig. 2) revealed that the mean redness (a*), yellowness (b*), and lightness (L*) were significantly higher (P < 0.0001) in the cooked shrimp than in the uncooked shrimp for all days tested. The standard deviations of the mean redness values of the cooked shrimp (6.28 ± 3.66) and the uncooked shrimp (1.19 ± 0.40) were large, indicating a wide variation in pink coloration for all days tested.

Effect of no added sodium tripolyphosphate on the pink color change of cooked shrimp. Color readings were taken before and after cooking for each shrimp that had not been treated with sodium tripolyphosphate and were analyzed after cooking. The color analysis (Fig. 3) revealed that the mean redness, yellowness, and lightness were significantly higher (P < 0.0001) in the cooked shrimp than in the uncooked shrimp for all days tested. The standard deviations of the mean redness values of the cooked shrimp (10.40 ± 2.24) and the uncooked shrimp (1.03 ± 0.93) were large, indicating a wide variation in pink coloration for all days tested. There were no significant differences in color change between cooked shrimp treated or not treated with sodium tripolyphosphate.

DISCUSSION

Because of its health benefits and flavorful taste, shrimp is in high demand in the United States. Unfortunately, an increase in consumption has resulted in increased disease outbreaks associated with shrimp consumed in restaurants and private residences. One of the main causes for the increase in outbreaks is believed to be inadequate cooking. Boiling is a popular cooking choice, and according to most cookbook recommendations, shrimp is considered “done” when it has floated to the surface of the water and is “pink” in color. The purpose of this study was to use scientific methods to determine whether this common method of cooking destroys the primary pathogens associated with shrimp.

TABLE 3. Growth of Salmonella Typhimurium, Salmonella Infantis, Salmonella Enteritidis during refrigerated storage of shrimp after they were boiled until floating

<table>
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<th>Salmonella inoculum</th>
<th>Cooked</th>
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<td></td>
<td>Day 0</td>
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<tr>
<td>Typhimurium</td>
<td>NDa</td>
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<tr>
<td>Infantis</td>
<td>ND</td>
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<td>Enteritidis</td>
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a ND, nondetectable (<10 CFU/g).
In the present study, boiling shrimp until they float was effective for significantly reducing *Listeria* and *Salmonella*. Initial bacterial loads were 3.5 to 5.4 log CFU/g per shrimp. After boiling until the shrimp floated, all pathogens were reduced to nondetectable levels, making the shrimp safe for consumption. No heat resistance was observed in any of the bacterial strains on the boiled shrimp, and no growth of any of the strains was detected during refrigerated storage of the shrimp that had been boiled until floating.

In previous studies, the ability of *L. monocytogenes* to grow on cooked shrimp stored at 4°C has been reported (17). This characteristic has serious implications for the food industry because if this pathogen were present in cooked shrimp, even at a low level, a dangerous level could be reached if these shrimp were stored at temperatures commonly used in chilled display cabinets at retail outlets (18). The findings of the present study indicate that boiling until floating eliminates *L. monocytogenes* in these shrimp to a level low enough that this pathogen is unable to grow or be detected, even after refrigerated storage.

The FDA and U.S. Department of Agriculture both recommend that seafood should be cooked until it reaches an internal temperature of 62.7°C (145°F) (15, 16). This recommendation is based on elimination of *L. monocytogenes*, which is the target pathogen associated with food because it is the most heat-resistant pathogen that does not form spores (15). In the present study, after shrimp were boiled until floating the minimum internal temperature of 68°C was still higher than the FDA’s recommended temperature. Because the mean surface temperature had a large standard deviation compared with that for the internal temperature, this measure was considered a less reliable source for determining doneness when cooking shrimp. After 50 s of boiling at 100°C, the shrimp reached an internal temperature of approximately 62°C, which is very close to the minimum safe internal temperature of 62.7°C recommended by the FDA for cooking seafood (15, 16). However, at 62°C the pathogenic bacteria had not been completely eliminated from the shrimp. Because of the high level of the inoculum, which is not typical for raw shrimp, survivors were detected; however, an ~5-log reduction was observed at 62°C. These results indicate that shrimp should be boiled to a minimum internal temperature of 68°C to ensure the elimination of pathogens and, consequently, safety for the consumer.

The National Advisory Committee on Microbiological Criteria for Foods (10) stated that “there is no single temperature, with or without specified cooking time, that will ensure the safety of all cooked fishery products,” and there are limited thermal inactivation data currently available for seafood-associated pathogens across various fishery products. In this study, the internal and surface

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<td>Day 0</td>
<td>Day 1</td>
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<tr>
<td><em>L. welshimeri</em></td>
<td>NDa</td>
<td>ND</td>
</tr>
<tr>
<td><em>L. monocytogenes</em></td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td><em>L. innocua</em></td>
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a ND, nondetectable (<10 CFU/g).

**FIGURE 2.** Comparison of mean L*, a*, and b* values on days 0, 1, and 2 for cooked and uncooked shrimp. Bars not connected by the same letter are significantly different (P < 0.0001, α = 0.05).

**FIGURE 3.** Comparison of mean L*, a*, and b* values on days 0, 1, and 2 for cooked and uncooked shrimp not treated with sodium tripolyphosphate. Bars not connected by the same letter are significantly different (P < 0.0001, α = 0.05).
temperatures of boiled shrimp were measured immediately after they floated. The minimum and mean (± SD) internal temperatures of the boiled shrimp were 65.2°C, and 68.61 ± 2.29°C, respectively. The mean (± SD) surface temperature of the boiled shrimp was 76.67 ± 4.9°C and the minimum surface temperature was 69.41°C. The wide degree of variation in internal and surface temperatures of the shrimp was the result of the different sizes of the shrimp. The smaller the shrimp, the faster it floats and vice versa. As a result, boiling the shrimp until it floats is a more reliable and easier way for the consumer to determine doneness than is measuring the temperature of the shrimp.

In addition, because the redness of shrimp is often used as an indicator of doneness in cooked shrimp with or without sodium tripolyphosphate, the redness was measured with a spectrophotometer, and the correlation between pathogen reduction to nondetectable levels and color change after boiling until floating was calculated. The redness of the cooked shrimp was significantly higher (P < 0.0001) than that of the uncooked shrimp. However, there was a large standard deviation in the redness values of the cooked shrimp, indicating a significant variation in the red color of these shrimp on all days tested.

The results of this study indicate that boiling shrimp until they float is an adequate method of reducing Listeria and Salmonella to levels safe for consumption. However, color change is not a reliable indication of doneness because of the high degree of variation in the red color of cooked shrimp. These results provide scientific evidence that boiling shrimp until they float is a dependable method for ensuring safe shrimp. This method can be easily understood and used by the consumer in a home setting.

ACKNOWLEDGMENTS

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REFERENCES